

CLAIMS

We claim:

5 1. An isolated bacterial strain, *Pseudomonas fluorescens* Biotype B E34, wherein the bacterial strain inhibits or arrests grassy weed germination.

10 2. An isolated bacterial strain, *Pseudomonas fluorescens* Biotype C WH19, wherein the bacterial strain inhibits or arrests grassy weed germination.

15 3. An isolated bacterial strain, *Pseudomonas fluorescens* Biotype C WH6, wherein the bacterial strain inhibits or arrests grassy weed germination.

20 4. An isolated bacterial strain, *Pseudomonas putida* Biotype B AH4, wherein the bacterial strain inhibits or arrests grassy weed germination.

25 5. An isolated bacterial strain, *Pseudomonas putida* Biotype B AD31, wherein the bacterial strain inhibits or arrests grassy weed germination.

30 6. A Germination-Arrest Factor, wherein the factor is produced by *Pseudomonas fluorescens* Biotype B E34, *Pseudomonas fluorescens* Biotype C WH19, *Pseudomonas fluorescens* C Biotype WH6, *Pseudomonas putida* Biotype B AH4, or *Pseudomonas putida* Biotype B AD31, wherein the Germination-Arrest Factor inhibits or arrests grassy weed germination.

35 7. The Germination-Arrest Factor of claim 6, wherein the grassy weed is *Poa annua*, *Poa trivialis* or *Bromus tectorum*.

8. The Germination-Arrest Factor of claim 6, wherein the grassy weed is crabgrass, goosegrass, dallisgrass, bahiagrass, annual bluegrass, downy brome, jointed goatgrass, roughstalk bluegrass, ratail fescue, perennial ryegrass, or tall fescue.

9. The Germination-Arrest Factor of claim 6, wherein the Germination-Arrest Factor is a hydrophilic molecule.

35 10. The Germination-Arrest Factor of claim 6, wherein the Germination-Arrest Factor has a molecular weight less than 3,000 daltons.

11. The Germination-Arrest Factor of claim 6, wherein the Germination-Arrest Factor reacts with ninhydrin.

12. The Germination-Arrest Factor of claim 6, wherein the Germination-Arrest Factor comprises an ionizable group.

5 13. An isolated nucleic acid as set forth in:

- (a) SEQ ID NO: 2;
- (b) SEQ ID NO: 7;
- (c) SEQ ID NO: 10; or
- (d) sequences having at least 90% sequence identity to (a), (b), or (c);

10 wherein the nucleic acid encodes a Germination-Arrest Factor or a protein involved in the synthesis and or secretion of a Germination-Arrest Factor.

14. An isolated Germination-Arrest Factor protein comprising an amino acid sequence as set forth in:

15 (a) SEQ ID NO: 3;

(b) SEQ ID NO: 4;

(c) SEQ ID NO: 8;

(d) SEQ ID NO: 11;

(e) SEQ ID NO: 12;

(f) SEQ ID NO: 13;

- (g) sequences having at least 90% sequence identity to (a), (b), (c), (d), (e), or (f); or
- (h) conservative variants of (a), (b), (c), (d), (e), or (f);

20 wherein the Germination-Arrest Factor protein inhibits or arrests germination in grassy weeds or is involved in the synthesis or secretion of a Germination Arrest Factor.

25 15. The Germination-Arrest Factor protein of claim 14, wherein the grassy weed is *Poa annua*, *Poa trivialis* or *Bromus tectorum*.

30 16. The Germination-Arrest Factor protein of claim 14, wherein the grassy weed is crabgrass, goosegrass, dallisgrass, bahiagrass, annual bluegrass, downy brome, jointed goatgrass, roughstalk bluegrass, ratail fescue, perennial ryegrass, or tall fescue.

35 17. The protein of claim 14, comprising the amino acid sequence as set forth in SEQ ID NO: 3.

18. The protein of claim 14, comprising the amino acid sequence as set forth in SEQ ID NO: 4.

19. The protein of claim 14, comprising the amino acid sequence as set forth in SEQ ID NO: 8.

20. The protein of claim 14, comprising the amino acid sequence as set forth in SEQ ID 5 NO: 11.

21. The protein of claim 14, comprising the amino acid sequence as set forth in SEQ ID NO: 12.

10 22. A method of inhibiting or arresting weed germination in a growth medium in which it would be desirable to inhibit or arrest grassy weed germination, the method comprising applying *Pseudomonas fluorescens* Biotype B E34, *Pseudomonas fluorescens* Biotype C WH19, *Pseudomonas fluorescens* C Biotype WH6, *Pseudomonas putida* Biotype B AH4, or *Pseudomonas putida* Biotype B AD31, or mixtures thereof to the growth medium in an amount sufficient to inhibit or arrest grassy 15 weed germination.

20 23. A method of inhibiting or arresting weed germination in a growth medium in which it would be desirable to inhibit or arrest grassy weed germination, the method comprising applying the Germination-Arrest Factor of claim 6 to the growth medium in an amount sufficient to inhibit or arrest grassy weed germination.

25 24. The method of claim 23, wherein the Germination-Arrest Factor is applied in a formulation that also comprises a surfactant, a stabilizer, a buffer, a preservative, an antioxidant, an extender, a solvent, an emulsifier, an invert emulsifier, a spreader, a sticker, a penetrant, a foaming agent, an anti-foaming agent, a thickener, a safener, a compatibility agent, a crop oil concentrate, a viscosity regulator, a binder, a tackifier, a drift control agent, a fertilizer, an antibiotic, a fungicide, a nematicide, or a pesticide.

30 25. The method of claim 23, wherein the Germination-Arrest Factor is applied in a formulation that is a solution, a soluble powder, an emulsifiable concentrate, a wettable powder, a liquid flowable, a dry flowable, a water-dispersible granule, a granule, or a pellet.

35 26. A method of inhibiting or arresting weed germination in grass seed, the method comprising applying *Pseudomonas fluorescens* Biotype B E34, *Pseudomonas fluorescens* Biotype C WH19, *Pseudomonas fluorescens* C Biotype WH6, *Pseudomonas putida* Biotype B AH4, or *Pseudomonas putida* Biotype B AD31 to the grass seed in an amount sufficient to inhibit or arrest grassy weed germination.

27. A method of inhibiting or arresting weed germination in grass seed, the method comprising applying the Germination-Arrest Factor of claim 6 to the grass seed in an amount sufficient to inhibit or arrest grassy weed germination.

5 28. The method of claim 27, wherein the Germination-Arrest Factor is applied in a formulation that also comprises a surfactant, a stabilizer, a buffer, a preservative, an antioxidant, an extender, a solvent, an emulsifier, an invert emulsifier, a spreader, a sticker, a penetrant, a foaming agent, an anti-foaming agent, a thickener, a safener, a compatibility agent, a crop oil concentrate, a viscosity regulator, a binder, a tackifier, a drift control agent, a fertilizer, an antibiotic, a fungicide, a 10 nematicide, or a pesticide.

15 29. The method of claim 27, wherein the Germination-Arrest Factor is applied in a formulation that is a solution, a soluble powder, an emulsifiable concentrate, a wettable powder, a liquid flowable, a dry flowable, a water-dispersible granule, a granule, or a pellet.

30. A composition for inhibiting or arresting the germination of grassy weeds, comprising:
the Germination-Arrest Factor of claim 6; and
a timed- or temperature-release coating over at least a portion of the Germination-Arrest
20 Factor.

31. The composition of claim 30, further comprising a water-resistant coating over the timed- or temperature-release coating.

25 32. A method of inhibiting or arresting weed germination in an area in which inhibiting or arresting weed germination is desirable, comprising:
broadcasting an herbicidally effective amount of the Germination-Arrest Factor of claim 6 at least once a year across the area, thereby inhibiting or arresting weed germination in the area.

30 33. The method of claim 32, wherein the area is a grass patch, an agricultural field, a natural landscape, or a road-side.

35 34. The method of claim 32, wherein the Germination-Arrest Factor is applied in a formulation that also comprises a surfactant, a stabilizer, a buffer, a preservative, an antioxidant, an extender, a solvent, an emulsifier, an invert emulsifier, a spreader, a sticker, a penetrant, a foaming agent, an anti-foaming agent, a thickener, a safener, a compatibility agent, a crop oil concentrate, a viscosity regulator, a binder, a tackifier, a drift control agent, a fertilizer, an antibiotic, a fungicide, a nematicide, or a pesticide.

35. The method of claim 32, wherein the Germination-Arrest Factor is applied in a formulation that is a solution, a soluble powder, an emulsifiable concentrate, a wettable powder, a liquid flowable, a dry flowable, a water-dispersible granule, a granule, or a pellet.

5 36. The method of claim 35, wherein the Germination-Arrest Factor is formulated as a granule.

37. The method of claim 36, wherein the granule is at least partially coated with a timed-or temperature-release coating.

10 38. The method of claim 37, wherein the timed-or temperature-release coating is coated with a water-resistant coating.

39. The method of claim 32, wherein the method is a method of inhibiting grassy weeds among dicot species.

15 40. A method of producing the Germination-Arrest Factor of claim 6 comprising: culturing *Pseudomonas fluorescens* Biotype B E34 in a suitable culture medium; collecting the culture medium; and purifying the culture medium to produce the Germination-Arrest Factor.

20 41. A method of producing the Germination-Arrest Factor of claim 6 comprising: culturing *Pseudomonas fluorescens* Biotype C WH19 in a suitable culture medium; collecting the culture medium; and purifying the culture medium to produce the Germination-Arrest Factor.

25 42. A method of producing the Germination-Arrest Factor of claim 6 comprising: culturing *Pseudomonas fluorescens* C Biotype WH6 in a suitable culture medium; collecting the culture medium; and purifying the culture medium to produce the Germination-Arrest Factor.

30 43. A method of producing the Germination-Arrest Factor of claim 6 comprising: culturing *Pseudomonas putida* Biotype B AH4 in a suitable culture medium; collecting the culture medium; and purifying the culture medium to produce the Germination-Arrest Factor.

35 44. A method of producing the Germination-Arrest Factor of claim 6 comprising: culturing *Pseudomonas putida* Biotype B AD31 in a suitable culture medium; collecting the culture medium; and

purifying the culture medium to produce the Germination-Arrest Factor.

45. A kit for inhibiting or arresting grassy weed growth, comprising:
the Germination-Arrest Factor of claim 6; and
5 a container.

46. The kit of claim 45, further comprising instructions for using the kit.

47. Germination-Arrest Factor for use as a seed-cleaning adjuvant in seed-cleaning
10 processes as a supplement or alternative to physical removal of target weed seeds.

48. A method of using Germination-Arrest Factor to investigate regulation of seed
germination and seedling development comprising using Germination-Arrest Factor to probe for
regulatory sites in plant cells and regulatory mechanisms controlling seed germination and
15 development.

49. A *Pseudomonas fluorescens* bacterial strain having the GAF-producing
characteristics of *Pseudomonas fluorescens* Biotype B E34 (deposited as NRRL # B-30481),
Pseudomonas fluorescens Biotype C WH19 (deposited as NRRL # B-30484), *Pseudomonas*
20 *fluorescens* C Biotype WH6 (deposited as NRRL # B-30485), *Pseudomonas putida* Biotype B AH4
(deposited as NRRL # B-30482), or *Pseudomonas putida* Biotype B AD31 (deposited as NRRL # B-
30483).

50. A Germination-Arrest Factor produced by the bacterial strain of claim 49, wherein
25 Germination-Arrest Factor is a hydrophilic molecule, has a molecular weight less than 3,000 daltons,
reacts with ninhydrin, and comprises an ionizable group.

51. A *Pseudomonas putida* bacterial strain having the GAF-producing characteristics
of *Pseudomonas fluorescens* Biotype B E34 (deposited as NRRL # B-30481), *Pseudomonas*
30 *fluorescens* Biotype C WH19 (deposited as NRRL # B-30484), *Pseudomonas fluorescens* C Biotype
WH6 (deposited as NRRL # B-30485), *Pseudomonas putida* Biotype B AH4 (deposited as NRRL #
B-30482), or *Pseudomonas putida* Biotype B AD31 (deposited as NRRL # B-30483).

52. A Germination-Arrest Factor produced by the bacterial strain of claim 49, wherein
35 Germination-Arrest Factor is a hydrophilic molecule, has a molecular weight less than 3,000 daltons,
reacts with ninhydrin, and comprises an ionizable group.